

Spatial and Diel Variability in Photosynthetic and Photoprotective Pigments in Shallow Benthic Communities

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LONG-TERM GOALS

Our overall goal is to understand how photosynthetic and photoprotective pigments in benthic plants (primarily benthic microalgae) affect the optical properties (primarily spectral reflectance and fluorescence) of shallow benthic environments. The information gained will be used for the development and testing of rapid scanning optical techniques for detecting and assessing changes and specific disturbances in benthic communities.

OBJECTIVES

Our main objective is to determine the spatial and temporal (particularly diel) variation in a variety of photosynthetic and photoprotective pigments and examine how these pigments affect the spectral reflectance and fluorescence at the sediment surface. An understanding of these relationships is needed in order to refine algorithms used for processing data collected with various multispectral and hyperspectral imaging instruments used for identification and characterization of both living and man-made objects in shallow benthic environments.

APPROACH

Laboratory studies are used to examine diel variation in the pigments of individual species of algae. We collect sediment and water samples over 24 hour cycles in a variety of types of environments, including various carbonate sediments around Lee Stocking Island, Bahamas and siliceous sands in Monterey Bay, California. Photosynthetic and photoprotective pigments are quantified in these samples using High Performance Liquid Chromatography (HPLC). Working with Charles Mazel, spectral reflection and fluorescence at the surface of sediment cores are measured. Changes in pigments over diel cycles are related to field measurements of spectral reflectance, absorbance and fluorescence.

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WORK COMPLETED

A new HPLC system with a diode array detector was set up for pigment analysis. This allows us to determine the absorption spectrum of individual pigments as they are eluted, and to use these spectra to verify pigment identities. In addition, we can simultaneously record absorption at 5 discrete wavebands for each pigment peak on the chromatograms. This latter capability is especially valuable for pigments present in low concentrations because it increases our ability to both detect and quantify these pigments based on the optimum absorption waveband for each pigment. In addition, we have modified the solvent gradient so that pigments of interest are separated from each other and from other unidentified absorbing compounds found in marine sediments with a single sample injection. For example, we are now able to completely separate zeaxanthin (found in both cyanobacteria and chlorophyte algae) from lutein (found in chlorophytes, but not in cyanobacteria) and therefore can estimate the contribution of each to total photosynthetic organism biomass as determined by chlorophyll a. The modifications we have made to the column temperature and solvent gradient protocols, and the addition of a diode array detector allow us to accurately quantify both major and minor lipid-soluble photosynthetic and photoprotective pigments using a single sample injection. This is a significant improvement over previously published methods. This new system has now been calibrated with pure pigments and tested with both algal cultures and field samples of both water and sediment.

Laboratory studies have been conducted on the diel variation in pigments, including xanthophyll cycling, in various species of algae.

Forty-seven sediment samples and 35 water column samples from various locations at Lee Stocking Island have been analyzed. Samples from Monterey Bay have been collected and are currently being processed.

Spectral reflectance measurements were made at the surface of sediment cores taken from three types of sediments at Lee Stocking Island. In addition, spectral reflectance measurements were made over diel cycles (three days duration) at the surface of sediments collected at a near-shore site.

Stimulated fluorescence (excitation and emission spectra) were measured at the surface of various types of sediments from Lee Stocking Island.

RESULTS

We have documented the diel variation in a number of pigments and the diel xanthophyll cycling in various species of algae.

Amounts and types of photosynthetic and photoprotective pigments varied dramatically among sediment types collected from various areas around Lee Stocking Island. Mean chlorophyll a, a measurement of plant biomass, varied approximately 50-fold, from around $0.3 \mu\text{g}/\text{cm}^2$ in the ooid shoals to over $15 \mu\text{g}/\text{cm}^2$ in the yellow grapestone sediments. Chlorophyll breakdown products are important components of many sediments because they, like their parent pigments, absorb in both the blue and red regions of the spectrum and so can profoundly affect optical properties of the sediments. At Lee Stocking Island, however, only samples in the yellow grapestone sediments contained large amounts of chlorophyll a breakdown products. Fucoxanthin, which absorbs in the blue and green

regions of the spectrum, was the most abundant carotenoid in all of the sediment samples. Fucoxanthin varied approximately 70-fold ($0.1 \mu\text{g} / \text{cm}^2$ in the ooid shoals and slightly greater than $7 \mu\text{g} / \text{cm}^2$ in the yellow grapestone sediments). Chlorophyll b, another pigment that absorbs in both the blue and red regions of the spectrum was not detectable in the ooid shoals and only small amounts (0.007 - $0.27 \mu\text{g} / \text{cm}^2$) were detected elsewhere. Zeaxanthin, which absorbs mostly in the blue region of the spectrum, was found at all sites in low concentrations (0.01 - $0.38 \mu\text{g} / \text{cm}^2$). Its presence, however, combined with the absence of chlorophyll b indicates that cyanobacteria are significant contributors to optical signals in the ooid shoals. The presence of both zeaxanthin and chlorophyll b at the other sites indicates that both chlorophytes and cyanobacteria are present at or near the sediment surface. In summary, these combined pigment data suggest that the ooid shoals and yellow grapestone sediments constitute end members for sediment modeling purposes near Lee Stocking Island.

In addition to the variability in pigment concentrations among the various sampling sites, small scale variabilities were apparent within sampling sites. The magnitude of the within site variability is illustrated by chlorophyll a, but it should be noted that superimposed on the variability of this one pigment is variability in chlorophyll:carotenoid ratios as well. At the North Perry site, two visually distinctive areas were observed, one with a yellow film on the sediment surface and one with little or no yellow film. Chlorophyll a measured on the surface of three cores varied from 2.8 to $4.8 \mu\text{g} / \text{cm}^2$ in the area with the film and 0.3 to $1.3 \mu\text{g} / \text{cm}^2$ in the area with little or no film. In the ooid shoals, chlorophyll a ranged from 0.2 to $0.3 \mu\text{g} / \text{cm}^2$ in the troughs and 0.4 to $0.7 \mu\text{g} / \text{cm}^2$ on the crests. Three areas were studied in the Rainbow Gardens area. The first was near coral (3.0 - $7.5 \mu\text{g} / \text{cm}^2$), the second was bare sand (3.0 - $4.3 \mu\text{g} / \text{cm}^2$), and the third was near seagrass (1.7 - $5.1 \mu\text{g} / \text{cm}^2$). Two types of sediments were noted at the Grapestone site, one white (0.5 - $0.6 \mu\text{g} / \text{cm}^2$) and the other yellow (14.3 - $16.7 \mu\text{g} / \text{cm}^2$). Norman's Cay Pond was quite variable, with chlorophyll a ranging from 6.7 to $12.7 \mu\text{g} / \text{cm}^2$.

Spectral reflectance measured at the surface of sediment cores from various types of environments around Lee Stocking Island indicate that each environment has unique reflectance properties. Not only is the amplitude of reflectance curves variable, but shapes of spectral reflectance curves vary as well. The slopes of the curves measured on various sediment types are particularly different from one another between 400 and 540 nm . The portions of the reflectance curves between 540 and 640 nm are more or less flattened, with the spectra of some sediment types being more complex in detail. All sediments were alike in that there was a distinct dip between 630 and 690 nm in reflectance. This feature was more or less pronounced, depending upon the amount of chlorophyll in the sediment. Diel changes in spectral reflectance were also noticable. The magnitude of reflectance decreased over all regions of the visible spectrum during the day. Slopes of the reflectance curves in the region between 400 and 540 nm decreased during the daytime and a peak in reflectance around 550 nm became more pronounced as the day progressed.

Spectral fluorescence measurements made on the surface of sediment cores revealed strong fluorescence emission around 680 nm , indicating the presence of chlorophyll a, and fluorescence at 580 nm , indicating the presence of phycoerythrin, at all study sites. These fluorescence signals varied significantly from site to site.

IMPACT/APPLICATIONS

The information generated will be used to enhance rapid remote sensing techniques for characterizing shallow benthic communities.

TRANSITIONS

The results of our field studies will be used by other members of the CoBOP team and other research and development programs within DOD concerned with remote sensing and underwater imaging, especially for optically shallow water environments. Measurements of spatial and temporal variations in plant pigments from both sediments and the water column will be used to define relationships between measured benthic and water column optical properties and to understand how optical properties are affected by biological, chemical and physical processes.

RELATED PROJECTS

In a project funded by NOAA, we are examining the spatial and temporal distribution of benthic microalgae (estimated by chlorophyll concentrations) in Florida Bay and their relationship to nutrient and phytoplankton distributions.

REFERENCES

None